Journal of Medical Hypotheses and Ideas (2015) **9**, 99–103

Available online at www.sciencedirect.com

Journal of Medical Hypotheses and Ideas

journal homepage: www.elsevier.com/locate/jmhi

REGULAR ARTICLE

Reprogramming of adult stem/progenitor cells into iPSCs without reprogramming factors

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Received 27 July 2015; revised 21 September 2015; accepted 22 September 2015
Available online 30 September 2015

KEYWORDS

Stem cells;
Cellular reprogramming;
Progenitor cells;
Induced pluripotency

Abstract Reprogramming of adult somatic cells into induced pluripotent stem cells (iPSCs) has attracted considerable attention in both the scientific and public communities. This is due to the importance of iPSCs in drug screening, disease modeling, cell transplantation therapies and regenerative medicine. A lot of efforts have been devoted to the generation of iPSCs with fewer reprogramming factors and with higher efficiencies. It has been shown that removal of reprogramming barriers increases the efficiency of iPSC generation from differentiated cells up to 90%. Interestingly, having relatively fast cell cycle kinetics, plasticity and endogenous expression of particular pluripotency regulators make adult stem/progenitor cells potentially elite cells poised to become iPSCs. Moreover, it has been demonstrated that adult stem/progenitor cells are more amenable to pluripotent reprogramming than mature cells. Accordingly, it is hypothesized that certain adult stem cells could be reprogrammed into iPSCs without overexpression of exogenous pluripotency transcription factors by only combinatorial modulation of barriers and enhancers and relying on the endogenous expression of key reprogramming factors (e.g. Oct4, Sox2, etc.).

Introduction

Somatic cells from different species (e.g. mouse [1–4], rat [5,6], monkey [7] and human [8–11]) have been reprogrammed into iPSCs by overexpression of pluripotency transcription factors most commonly Oct4, Sox2, c-Myc and Klf4 (OSKM) in mouse, and Oct4, Sox2, Nanog and Lin28 in human cells. Human iPSCs are an important alternative cell source for patient-specific cell-based therapies due to their ability for unlimited self-renewal and pluripotent differentiation. Interestingly, derivatives of pluripotent stem cells have been introduced to the clinic, representing the promise of a new

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URL: www.tums.ac.ir/english/
doi:http://dx.doi.org/10.1016/j.jmhi.2015.09.003

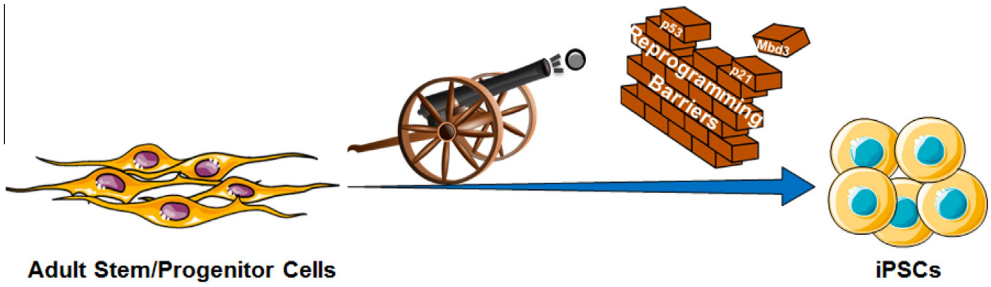

Fig. 1 A schematic representation of the proposed theory. The removal of reprogramming barriers could enable adult stem/progenitor cells to be reprogrammed into iPSCs without ectopic expression of exogenous factors relying on the endogenous expression of pluripotency master regulators and appropriate extracellular cues.

era for regenerative medicine [12–15]. However, the genetic manipulation of reprogrammed cells limits their applications in regenerative medicine. This issue highlights the importance of integration-free reprogramming methods for the generation of iPSCs. Moreover, pluripotent reprogramming is an inefficient process due to various defined and undefined barriers [16]. Indeed, the main drawback of reprogramming is its low efficiency [17]. It has been assumed that the somatic programs or somatic gene regulatory networks (GRNs) protect the cells from aberrant transformations and provide barriers to an efficient reprogramming [18–20]. Pluripotent reprogramming should overcome the epigenetic state of a differentiated somatic cell to make it compatible with the pluripotent state. Thus, an important factor affecting the efficiency of reprogramming is the differentiation state of the starting (donor) cells, which acts as a barrier to efficient epigenetic remodeling of the genome [21]. Different barriers of reprogramming (p53, p21, p57, $p16^{Ink4a}$/$p19^{Arf}$, Mbd3, etc.) have comprehensively reviewed elsewhere [16]. It has been demonstrated that removal of the main barriers of somatic cell reprogramming or combinatorial modulation of barriers and enhancers dramatically improves the efficiency of the process to nearly 100% [22,23]. However, somatic programs and epigenetic barriers of reprogramming seem to be more strict in differentiated cells than stem/progenitor cells, which are in a plastic state [21]. Adult stem cells are undifferentiated cells with plasticity and self-renewal capacity [24,25]. Interestingly, adult stem and progenitor cells have certain similarities with pluripotent stem cells, such as the ability to differentiate into distinct cell types and the expression of specific pluripotency regulators [26,27]. Furthermore, different studies have confirmed that adult stem/progenitor cells are more amenable to reprogramming than mature cells and can be efficiently reprogrammed into iPSCs [21,28–32]. This study considers the possibility of reprogramming of adult stem cells into iPSCs without ectopic expression of pluripotency transcription factors by only depletion of barriers and activation of enhancers (Fig. 1).

The hypotheses/ideas

Considerable efforts have been devoted to developing various methods for improving the reprogramming efficiency [16]. Surprisingly, it has been indicated that mouse iPSCs can be produced by a chemical cocktail and without reprogramming factors, but with delayed kinetics and low efficiency [33]. This finding shows that induction of pluripotency in mouse cells can be accomplished in the absence of reprogramming transcription factors; however, overexpression of reprogramming factor(s) still is essential for efficient induction of reprogramming [16]. Adult stem cells and progenitors are in a plastic state and express certain pluripotency regulators [25,34–36]. Moreover, findings have demonstrated that these cells are more amenable to reprogramming than differentiated cells [21]. It could be suggested that differences between pluripotency and multipotency arise from distinct genetic and epigenetic barriers, which lock multipotent stem cells in a restricted state of potency and prevent them from aberrant transformations to pluripotency and malignancies. In addition, stem and progenitor cells do not express lineage-specific genes, which can hamper reprogramming and in turn express certain embryonic stem (ES) cell markers [25,34–36], which could allow their efficient reprogramming. Therefore, the use of stem/progenitor cells that endogenously express appropriate levels of pluripotency factors as starting cells can reduce the number of reprogramming factors for iPSC production [29,37]. Consequently, it could be hypothesized that adult stem/progenitor cells can be simply reprogrammed into iPSCs without overexpression of reprogramming factors by only depletion of reprogramming barriers and application or activation of enhancers (Fig. 1).

Evaluation of the hypotheses/ideas

A large number of reprogramming barriers have been identified hitherto, including p53-p21 pathway, Wnt/β-catenin, TGF-β and Hippo signaling pathways, histone H3 Lysine 9 (H3K9) methylation, histone H3 Lysine 79 (H3K79) methylation, H3K36me2/3 marks, histone deacetylation and MBD3/NuRD complex [16]. Depletion or inhibition of these barriers has been successful to enhance reprogramming efficiency of somatic cells. Furthermore, activation of specific genes and pathways can accelerate the process [16].

Thus, for evaluating the applicability of the induction of pluripotency in adult stem/progenitor cells without overexpression of exogenous reprogramming factors, it is suggested that chemical or biological molecules (e.g. siRNAs or small molecules), which can inhibit barriers or activate enhancers either separately or together, are administered in cultures of

adult stem cells. Moreover, it is recommended that the cells are cultured in pluripotent-supportive reprogramming media.

Discussion

Different methods are developed for enhancing the efficiency of iPSC production. It has been shown that removal of reprogramming roadblocks facilitates and accelerates OSKM reprogramming function [16].

To achieve the minimum number of reprogramming factors, multiple studies have been done on differentiated cells. Morrisey and colleagues revealed that expression of the miR302/367 cluster in combination with Hdac2 suppression substitutes OSKM and rapidly and efficiently reprograms mouse and human somatic cells to iPSCs by activating endogenous Oct4 and its targets [38]. Wang et al. demonstrated that overexpression of high-performance synthetic OCT4-VP16 alone reprograms mouse embryonic fibroblasts (MEFs) into iPSCs [39]. Therefore, Oct4 activation seems essential for reprogramming.

Interestingly, pluripotency factor-induced transdifferentiation is a fate conversion approach that uses iPSC transcription factor(s) to induce an unstable or plastic cell state in mature cells [40–48]. The generated plastic cells by this approach become responsive to environmental cues and can be transdifferentiated toward various fates [40–48]. In order to minimize manipulations in this paradigm, it has been demonstrated that Oct4 alone in combination with short-term exposure to reprogramming medium is sufficient to induce a plastic state in human fibroblasts [44–46]. Surprisingly, Salci et al. showed that continued and prolonged (45–93 days) culture of human OCT4-induced plastic fibroblasts (plastic $hFib^{OCT4}$) in a pluripotent-supportive reprogramming media is sufficient for their pluripotent reprogramming [49]. The underlying mechanism of Oct4-induced plasticity remained to be elucidated, but a possible explanation for this kind of induced plasticity is Oct4-mediated extinguishment of native GRNs. Therefore, expression of Oct4 and disruption of the somatic GRNs are critical factors during successful reprogramming to pluripotency. Various methods of somatic cell reprogramming have indicated that the activity of exogenous or endogenous Oct4 is indispensable during the reprogramming process and there is no substitute that can replace Oct4's function in the absence of other reprogramming factors [49,50].

It has long been known that stem/progenitor cells (e.g. mesenchymal stem cells (MSCs)) express certain pluripotency regulators. For instance, MSCs from human bone marrow [34,35], adipose tissue, heart, dermis [34] and dental pulp [25,36] express certain key pluripotency genes (e.g. *Oct4*, *Nanog*, *Sox2*). Moreover, OCT4 has similar target genes and regulatory circuitries in human embryonic stem cells (hESCs) and human bone marrow mesenchymal stem cells (hBMSCs) [35]. Additionally, there is evidence that shows that tissue stem/progenitor cells could be initiators or origin of cancer due to their longevity and self-renewal capacity [51–53]. Furthermore, results from somatic cell nuclear transplantation have revealed that transferred nucleus of neural stem cells and keratinocyte stem cells produce cloned embryos more efficiently than differentiated cells [54,55]. Collectively, these findings represent adult stem cells as valuable sources for iPSC production.

To this end, several groups have endeavored to reprogram adult stem cells and progenitors with more efficiency or with fewer factors. For instance, it has been revealed that Oct4 and Sox2 can reprogram cord blood-derived $CD133^+$ stem cells into iPSCs, whereas they are unable to generate iPSCs from differentiated keratinocytes and fibroblasts [28]. Furthermore, Kim and colleagues showed that Oct4 alone is sufficient for the generation of iPSCs from mouse and human neural stem cells (NSCs), which endogenously express Sox2, c-Myc, Klf4 and SSEA1 [29,37]. Hochedlinger and colleagues demonstrated that differentiation stage of starting cells has an intense impact on the efficiency and kinetics of reprogramming, and that hematopoietic stem and progenitor cells can be reprogrammed 300 times more efficient than terminally differentiated cells. They demonstrated that hematopoietic stem/progenitor cells are more amenable to reprogramming than differentiated cell types because their epigenetic state is more permissible to transcription factor-induced remodeling [21]. Similarly, Park et al. revealed that human endometrial cells, which endogenously express elevated levels of pluripotency factors are more amenable to reprogramming to pluripotency than fibroblasts [56]. Moreover, Vidal et al. recently showed that specific progenitor cells have simpler requirements than fibroblasts for highly efficient and synchronous reprogramming [23].

These data are indicative of a "primed" state in stem/progenitor cells for efficient acquisition of pluripotency in defined conditions [23]. This property may be due to some intrinsic features of stem/progenitor cells, including expression of stemness related genes, permissible chromatin state, a decreased level of barriers (e.g. TGF-β and MAP kinase pathways) and increased levels of genetic and epigenetic facilitators (e.g. KDM2B) that favor reprogramming [23]. Possibly, differences between embryonic and adult stem cells can originate from specific intrinsic barriers, which in a fine tuning process regulate gene expression levels desired for the maintenance of pluripotency or multipotency.

There are data that demonstrate that (1) removal of barriers or activation of enhancers can increase the reprogramming efficiency of mature cells to 100% [22,23] (well discussed by Ebrahimi [16]), (2) stem/progenitor cells can be reprogrammed more efficiently than mature cells [21], (3) stem/progenitor cells can be reprogrammed with fewer factors [29,37], (4) combinatorial modulation of barriers and enhancers can improve reprogramming efficiency of stem/progenitor cells to nearly 100% [23]. According to these findings, it may be possible to convert stem/progenitor cells, which have a reservoir of reprogramming transcription factors, into iPSCs without exogenous expression of pluripotency factors by only depletion of reprogramming barriers and activation of enhancers (Fig. 1). Although this is an interesting concept, it still needs to be confirmed by experimental evidence.

Collectively, depletion of barriers and activation of enhancing pathways could be a very fast, inexpensive, feasible and accessible method for reprogramming of adult stem/progenitor cells into iPSCs. Clinically, this approach could be a safe and efficient method of iPSC derivation from tissues (e.g. dental pulp, adipose, bone marrow, etc.) taken from patients and provides promising hopes for stimulation of tissue-specific progenitor cells *in situ* to proliferate and regenerate injuries or

deficiencies. Moreover, generation of iPSCs without reprogramming factors can accelerate the clinical application of these cells and reprogramming technology in order to regenerative purposes.

Overview Box

First question: What do we already know about the subject?

Low efficiency of pluripotent reprogramming can be improved by removing reprogramming barriers. The findings demonstrate that adult stem cells are more amenable to reprogramming than differentiated cells, possibly due to endogenous expression of certain reprogramming factors. Suggestively, adult stem cells could be converted into iPSCs by only depletion of barriers and activation of enhancers relying on the endogenous expression of specific pluripotency factors and appropriate extracellular stimuli.

Second question: What does the proposed theory add to the current knowledge available, and what benefits does it have?

Generation of patient specific-iPSCs holds tremendous promise for the treatment of human disease. If the proposed theory which addressed here is confirmed by future investigations, safe, integration-free and clinical-grade iPSCs can be generated more efficiently by a simple non-integrating technique [57]. Ultimately, this theory offers a faithful and highly efficient reprogramming method that could provide powerful tools for research studies, disease modeling, drug screening and cell transplantation therapies.

Third question: Among numerous available studies, what special further study is proposed for testing the idea?

It is proposed that adult stem cells derived from various tissues (e.g. bone marrow, adipose, dental pulp, umbilical cord, etc.) are cultured in reprogramming medium containing factors capable of modulation of barriers (i.e. inhibition) and enhancers (i.e. activation).

Conflicts of interest

The author declares that there are no conflicts of interest.

Acknowledgement

I would like to thank Mohammad Hossein Soltani, and Mahdieh Namayandeh from Yazd Cardiovascular Research Center for their administrative supports.

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